REMARKS

Claims 28, 29, 31, 32, 34, 36-47, 55, 57, 58, and 62-66 are pending and have been examined. Claims 28, 29, 31, 32, 34, 36-47, 55, 57, 58, and 62-66 stand rejected. Claims 29, 32, 34, 36-47, 55, 57, 58, 62, and 64-66 have been canceled, and Claims 28, 31, and 63 have been amended. Applicant respectfully requests reconsideration and allowance of Claims 28, 31, and 63.

Rejection of Claims Under 35 U.S.C. § 112, Second Paragraph

The Examiner has rejected Claim 44 under 35 U.S.C. § 112, second paragraph, as being indefinite. Claim 44 has been canceled. Accordingly, this ground for rejection is now moot.

Rejection of Claims Under 35 U.S.C. § 112, First Paragraph (Enablement)

The Examiner has rejected Claims 28, 29, 31, 32, 34, 36-47, 55, 57, 58, and 62-66 under 35 U.S.C. § 112, second paragraph, as not being enabled by the specification. As a preliminary matter, applicant notes that the subject matter relating to $p21^{Cip1}$ and $p57^{Kip2}$ has been canceled from the claims. The pending claims are now directed to the use of antisense molecules directed to $p27^{Kip1}$ to treat hearing loss. Accordingly, the Examiner's rejections as they apply to $p21^{Cip1}$ and $p57^{Kip2}$ and inhibitors other than $p27^{Kip1}$ antisense molecules are moot. In addition, Claims 29, 32, 34, 36-47, 55, 57, 58, 62, and 64-66 have been canceled.

According to the Examiner, the experiments in the specification only demonstrate proliferation in response to inhibition of $p27^{Kip1}$ but do not show that the resulting cells are properly developed and provide viable sensory cells that result in a treatment for hearing loss such as perception deafness. Applicant respectfully disagrees. The specification states that cell division represents the decisive step in the hair cell regeneration process (specification, page 9, line 2), Moreover, the specification notes that the mitosis of the supporting cells in $p27^{Kip1}$ knockout mice results in mature sensory cells and that these knockout mice consequently have

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more hair cells than normal mice (specification, page 9, lines 6-9). Therefore, as pointed out in the specification, the experiments enable the conclusion to be drawn that in addition to cell division, "there is also differentiation or maturation to hair sensory cells and finally a functional recovery of auditory function of the sensory organ" (specification, page 9, lines 1-5).

The specification further describes that regeneration of sensory cells is possible even when $p27^{Kip1}$ activity is not completely eliminated because a gene dose-dependent effect on the regeneration of the sensory cells is observed in $p27^{Kip1}$ heterozygous mice (specification, page 9, lines 25-28). Thus, after destroying the hair cells by the systemic administration of the ototoxic antibiotic amikacin to $p27^{Kip1}$ heterozygous mice, examination of the cochlea revealed regenerated hair cells (specification, page 9, lines 10-18). Furthermore, appended hereto as Attachment A is the Second Declaration of Dr. Jonathan Kil ("the Second Kil Declaration"), which describes experiments carried out by Dr. Kil and his co-workers to assess the proliferation of supporting cells in the organ of Corti and the level of hair cell regeneration in response to aminoglycoside ototoxicity in $p27^{Kip1}$ homozygous mutant mice. Specifically, administration of amikacin to $p27^{Kip1}$ homozygous mutant mice resulted in increases in proliferation of many of the supporting cell types in the organ of Corti as well as hair cell regeneration (Second Kil Declaration, paragraphs 4-6, Table 1). In addition, the Second Kil Declaration describes experiments demonstrating that partial elimination of $p27^{Kip1}$ function in $p27^{Kip1}$ heterozygous mice results in an improvement in auditory function after treatment of the mice with amikacin (Second Kil Declaration, paragraph 7, Table 2, and Figure 1). Moreover, as acknowledged by the Examiner, the Declaration of Dr. Jonathan Kil appended to the amendment filed on March 7, 2003 ("the First Kil Declaration"), demonstrates that the local administration of $p27^{Kip1}$ antisense molecules, which result in a dose-dependant reduction of p27Kip1 mRNA and protein levels, causes proliferation of cells in the organ of Corti. In combination, the results described in

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the First Kil Declaration and the Second Kil Declaration show that reducing $p27^{Kip1}$ activity by locally administering $p27^{Kip1}$ antisense molecules results in proliferation of cells in the organ of Corti and that the partial elimination of $p27^{Kip1}$ function in $p27^{Kip1}$ heterozygous mice results in an improvement in auditory function after treatment of the mice with amikacin.

The Examiner further states that the specification does not provide specific guidance on the treatment of perception deafness because perceptive deafness encompasses a broad range of diseases and disorders, including for example deafness caused by destruction of sensory cells wherein the origin of the destruction is genetic, unrelated to cell cycle inhibitors. Applicant respectfully disagrees because the methods of the invention are not limited to a particular cause for the destruction of sensory cells that leads to hearing loss. Specifically, the methods of the invention are not directed to reversing the destruction of sensory cells but rather to stimulating the proliferation of the *supporting cells* present in the sensory epithelium, thereby promoting the regeneration of functional sensory cells (see specification, page 3, lines 12-22). Thus, the manner in which the sensory cells were destroyed to produce hearing loss, such as perception deafness, is not critical provided that there are viable supporting cells that can proliferate in response to the inhibition of $p27^{Kip1}$ activity.

For the reasons described above, applicant submits that the specification provides an enabling description for Claims 28, 31, and 63. Withdrawal of this ground of rejection is respectfully requested.

Rejection of Claims Under 35 U.S.C. § 112, First Paragraph (Written Description)

The Examiner has rejected Claims 28, 29, 31, 32, 34, 36-47, 55, 57, 58, and 62-66 under 35 U.S.C. § 112, second paragraph, as lacking an adequate written description. As described in the preceding section, the subject matter relating to $p21^{Cip1}$ and $p57^{Kip2}$ has been canceled from the claims. The pending claims are now directed to the use of antisense molecules directed to

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$p27^{Kip1}$. Accordingly, the Examiner's rejections as they apply to $p21^{Cip1}$ and $p57^{Kip2}$ and inhibitors other than $p27^{Kip1}$ antisense molecules are moot. In addition, Claims 29, 32, 34, 36-47, 55, 57, 58, 62, and 64-66 have been canceled.

Applicant respectfully submits that Claims 28, 31, and 63 are supported by an adequate written description. As noted by the Examiner, Coats et al. (1996) *Science* 272:877-880 and Hauser et al. (1997) *Cell Growth and Differentiation* 8:203-211, already of record, disclose antisense inhibitors for $p27^{Kip1}$. These antisense inhibitors of $p27^{Kip1}$ were known and used prior to the filing date of the present application. Therefore, one of skill in the art would recognize that the inventor possessed the invention of Claims 28, 31, and 63. Accordingly, withdrawal of this ground of rejection is respectfully requested.

Rejection of Claim Under 35 U.S.C. § 102(b)

The Examiner has rejected Claim 55 under 35 U.S.C. § 102(b) as being anticipated by Hauser et al., *Cell Growth and Differentiation* 8:203-11, 1997. Claim 55 has been canceled. Accordingly, this ground of rejection is now moot.

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CONCLUSION

In view of the foregoing claim amendments and remarks, applicant respectfully submits that Claims 28, 31, and 63 are in condition for allowance. Reconsideration and favorable action are requested.

Respectfully submitted,

CHRISTENSEN O'CONNOR
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I hereby certify that this correspondence is being deposited with the U.S. Postal Service in a sealed envelope as first class mail with postage thereon fully prepaid and addressed to Mail Stop AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the below date.

Date: 5/11/04 Carole Julyan

BFM:KBB/cj

MAIL STOP AF
RESPONSE UNDER 37 C.F.R. § 1.116
EXPEDITED PROCEDURE
EXAMINING GROUP 1600

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	H. Lowenheim	Attorney Docket No.: SOPH116953
Application No.:	09/622,719	Group Art Unit: 1635
Filed:	October 18, 2000	Examiner: K.A. Lacourciere
Title:	METHOD FOR THE TREATMENT OF DISEASE OR DISORDERS OF THE INNER EAR	

SECOND DECLARATION OF JONATHAN KIL

Seattle, Washington 98101

May 7, 2004

TO THE COMMISSIONER FOR PATENTS:

I, Dr. Jonathan Kil, declare as follows:

1. I am the CEO of Sound Pharmaceuticals, Inc., Seattle, Washington, and I am familiar with the subject matter disclosed and claimed in the above-identified application.

2. A copy of my *curriculum vitae* is appended hereto as Attachment B.

3. I have considered the Office Action dated February 13, 2004, issued in the above-identified application. It is my understanding that the Examiner has rejected claims in the application on the basis of lack of enablement. The Examiner has relied on Pfister & Löwenheim (2002) *Gentherapeutische Aspecte am Innenohr*, pp. 50-7 and Chen & Segil (1999) *Development* 126:1581-90 to conclude that it would take undue experimentation to control the development of sensory cells by administering an inhibitor of cell cycle inhibitors. In addition, the Examiner has relied on Löwenheim et al. (1999) *Proc. Natl. Acad. Sci. U.S.A.* 96:4084-8 to conclude that it is unclear whether release of cells from inhibition of proliferation would initiate the further events required to complete the hair cell regeneration process required to achieve a treatment effect for a disease or disorder of the inner ear.

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4. My colleagues and I conducted the following experiments (1) to assess the proliferation of supporting cells in the organ of Corti and the level of hair cell regeneration in response to aminoglycoside ototoxicity in $p27^{Kip1}$ homozygous mutant mice, and (2) to evaluate the improvement in auditory function in $p27^{Kip1}$ heterozygous mice treated with the ototoxic agent amikacin sulfate.

5. Mouse pups of $p27^{Kip1}$ homozygous null (-/-) received amikacin (1800 mg/kg) from P7 to P12 by subcutaneous (sc) injection once daily. The mitotic tracer 5-bromo-2-deoxyuridine (BrdU) (30 mg/kg, sc) was injected once daily from P10 to P12 simultaneously. Control mice received equivalent injections of BrdU only. Animals were sacrificed 2 days after the last amikacin and BrdU injection.

Mice were decapitated, the cochleae were dissected out and perfused with 4% paraformaldehyde through the round window, then immersed in the same fixative for another 30 minutes. After further dissection, the cochleae were treated with 0.3% hydrogen peroxide in 90% methanol to reduce endogenous peroxidase activity. Cochlea were reacted with 2N HCl to denature the DNA, blocked with 10% horse serum for 1 hour, and incubated with biotin-conjugated sheep anti-BrdU antibody (1:300, BioDesign) overnight at 4°C. After rinsing, the tissues were incubated with an ABC solution (Vector Laboratories), washed, then reacted with diaminobenzidine nickel chloride. Samples were post-fixed with osmium tetroxide (1% in 0.1 M sodium cacodylate buffer), dehydrated through an ethanol series, infiltrated with a mixture of ethanol and historesin, and embedded in historesin. Semi-thin sections (4 microns) were cut and collected. The sections were counter stained with toluidine blue and examined using DIC light microscopy. Each section was examined and each BrdU-positive nucleus was recorded. BrdU-positive nuclei in the same cell type and location in adjacent sections were excluded to avoid double counting.

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6. As shown in Table 1, cell proliferation in the organ of Corti of $p27^{Kip1}$-/- mice was increased after aminoglycoside ototoxicity. 831 sections from an amikacin/BrdU-treated cochlea and 990 sections from a BrdU-treated cochlea were analyzed individually. Cell type determination was based on both morphology and location within the organ of Corti. Normalized values reflect the number of cells expected in the organ of Corti based on 1000 sections at 4 micron thickness.

BrdU-positive nuclei were observed in both hair cells and supporting cells. The number of BrdU-positive inner hair cells (IHCs) and outer hair cells (OHCs) increased following amikacin treatment. An increase in proliferation was also observed in inner phalangeal, Deiter's, and Hensen cells. These data indicate that many of the supporting cell types in the organ of Corti exhibit increases in proliferation in response to amikacin treatment and that the level of hair cell regeneration also increases in response to aminoglycoside ototoxicity.

7. To measure the improvement in auditory function in amikacin sulfate-treated $p27^{Kip1}$ heterozygotes, mice (P7-P12) received systemic injections of amikacin sulfate (500mg/kg/d/s.c) for six consecutive days. Auditory function was measured two weeks and four weeks after amikacin sulfate treatment using the auditory brainstem response (ABR) using subcutaneous recording electrodes placed on three head points in isofluorane-anesthetized mice. The sound intensity threshold was determined by presenting single frequencies as different sound intensities (intensity measured in logarithmic scale or decibels). The higher the tone intensity that is required to elicit the ABR, the higher the auditory threshold, *i.e.*, the worse the auditory function. Significance is determined using one-way analysis of variance (ANOVA) for each stimulus frequency and intensity. Differences are considered statistically significant for p-values <0.05. The data set forth in Table 2 and FIGURE 1 shows auditory improvement in six $p27^{Kip1}$ heterozygote ears compared to four wild-type ears. These results clearly indicate that cells

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released from inhibition of proliferation as a consequence of decreasing $p27^{Kip1}$ gene dosage do initiate and complete the hair cell regeneration process required to achieve an auditory improvement after treatment with an ototoxic agent.

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Table 1. BrdU-Positive Nuclei of Different Cell Types in the Organ of Corti in $p27^{Kip1}$ Mice

Cell Type	Amikacin +BrdU		BrdU		
	# of BrdU Nuclei	Normalized	# of BrdU Nuclei	Normalized	Exp./Con.
Inner Phalangeal Cells	176	211.8	66	66.7	3.18
Inner Hair Cells	27	32.5	10	10.1	3.22
Pillar Cells	30	36.1	43	43.4	0.83
Outer Hair Cells	5	6	1	1	6
Deiter's Cells	9	10.8	5	5.1	2.12
Hensen Cells	128	154	30	30.3	5.08
Sum	375	451.3	155	156.6	2.88
Sections	831	1000	990	1000	

Table 2. ABR Thresholds at 4 Weeks Post-Amikacin Sulfate Treatment

	4kHz	8kHz	12kHz	16kHz	24kHz
Wildtype Ears					
1	95	85	80	85	90
2	90	95	95	95	90
3	95	85	80	85	90
4	100	95	110	90	90
Mean	95.00	90.00	91.25	88.75	90.00
St. Dev.	4.08	5.77	14.36	4.79	0.00
St. Error	2.04	2.89	7.18	2.39	0.00
$p27^{Kip1}$ Heterozygote Ears					
1	90	80	65	65	90
2	35	45	45	45	35
3	25	40	35	30	45
4	45	60	30	35	50
5	35	35	35	35	60
6	35	30	35	25	55
Mean	44.17	48.33	40.83	39.17	55.83
St. Dev.	23.33	18.62	12.81	14.29	18.82
St. Error	9.52	7.60	5.23	5.83	7.68
P	0.0029	0.0027	0.0004	0.0002	0.0074

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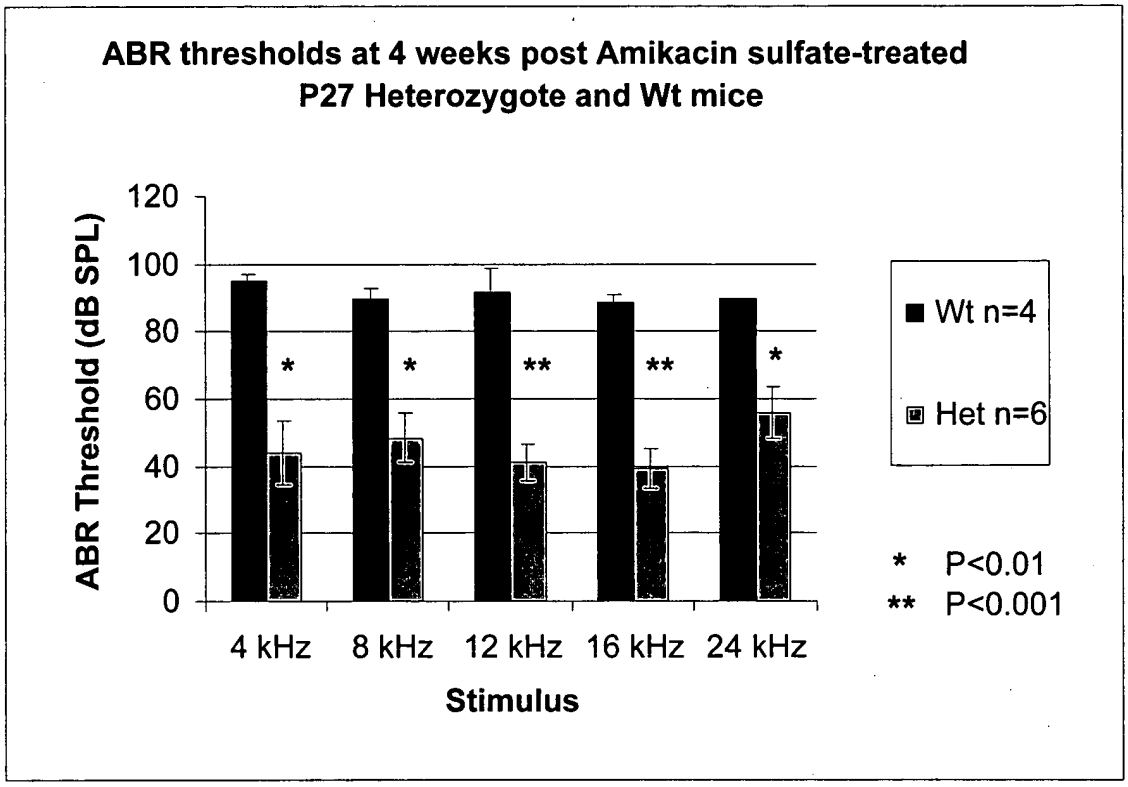
FIGURE 1



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8. All statements made herein and of my own knowledge are true, and all statements made on information and belief are believed to be true; and further, these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

Respectfully submitted,

Jonathan Kil, M.D.

Date: 5/6/04

KBB:KBB

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JONATHAN KIL, M.D.

Education:

University of Virginia	M.D.	1992-1996
Georgetown University	MD/PhD (candidate)	1990-1992
University of California, Irvine	B.S. in Biological Sciences	1985-1989

Experience:

2001-	Founder, President, CEO Sound Pharmaceuticals Inc., Seattle, WA
1998-2001	Founder, President, CEO, CSO Otogene USA, Inc., Seattle, WA Member of the Vorstand, Otogene AG, Tuebingen, Germany
1996-1998	Senior Fellow Department of Otolaryngology-HNS and VM Bloedel HRC University of Washington
1992-1996	M.D./Ph.D. (candidate) Departments of Neurosciences and Otolaryngology-HNS University of Virginia (UVA)
1990-1992	M.D./Ph.D. candidate (transferred to UVA to conduct inner ear research) Departments of Cell Biology and Otolaryngology-HNS Georgetown University
1989-1990	Research Assistant Department of Anatomy and Neurobiology University of California, Irvine

Grants:

2000-2002	NIH SBIR Phase II #DC04258-02, P.I.
1999-2000	NIH STTR Phase I #DC04258-01, P.I.
1996-1998	Individual NRSA Postdoctoral Research Fellowship #DC00247, P.I.
1991-1992	American Hearing Research Foundation Research Grant, Co-investigator

Awards/Honors:

1995	Association for Research in Otolaryngology Medical Student Travel Award
1995	Winn Medical Student Scholarship for Otolaryngology-HNS
1991	Achievement Reward for College Scientists (ARCS) Foundation Scholarship
1989	Ralph W. Gerard Award for Outstanding Research
1989	Excellence in Research Honors

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